REMARKS

This application has been amended in a manner that is believed to place it in condition for allowance at the time of the next Official Action.

Claims 1-2 and 4-22 are pending in the application. Claims 1 and 13 have been amended to address the formal matters raised in the outstanding Official Action.

At this time, applicants respectfully request that the withdrawn claims 13-21 be rejoined and considered. Claims 13-21 currently recite the elected invention and species of SEQ ID NOs: 9, 10, 13, 14, and 15. However, applicants note that claim 8 has already been examined with regards to SEQ ID NOs: 11 and 12. Accordingly, applicants ask that the sequences remain under consideration and submit that the Patent Office can not contend that there is a burden in examining these sequences. Thus, applicants respectfully request a search and examination of all of the claims in their full scope.

In the outstanding Official Action, claim 22 was objected to for allegedly being of improper dependent form for failing to further limit the subject matter of independent claim 1. However, claim 1 has been amended to recite that a total amount of amplicons is higher than the reference values indicative of the presence of "colorectal tumors or pre-cancerous lesions". Claim 22 is specifically directed to colorectal

tumors. Thus, applicants respectfully submit that claim 22 is a proper dependent claim of claim 1.

Claims 1, 2, 4-6, 8-11 and 21 were rejected under 35 USC 112, second paragraph, for allegedly omitting essential steps. This rejection is traversed.

Independent claim 1 has been amended to recite steps wherein pre-cancerous lesions are considered. Accordingly, applicants respectfully request that this rejection be withdrawn.

Claims 1, 4-6, 9-11 and 22 were rejected under 35 USC 102(b) as allegedly being anticipated by SHUBER. This rejection is traversed

As the Examiner is aware, a claim is anticipated only if each and every recitation as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Applicants submit that SHUBER fails to describe a method of method for determining the presence of colorectal tumors or pre-cancerous lesions in a human subject that utilizes a step of "amplifying by PCR at least three different DNA fragments with a length of 100 base pairs or more, using deoxynucleotide triphosphates or primers labelled with fluorescent molecule".

Contrary to the assertions of the Official Action, SHUBER never utilizes such a step for detecting colorectal

cancer. Rather, SHUBER's statement, "labels, such as fluorescent or radioactive labels, may be used" (page 8, 1st paragraph) generally refers to the methods of disease detection cited by SHUBER. There is no suggestion or example or utilizing such a step for a method of detecting colorectal cancer. Indeed, contrary to the assertions of the Official Action, SHUBER's statement "labels, such as fluorescent or radioactive labels, may be used" (page 8, 1st paragraph), does not suggest the fluorescent steps utilized by the claimed method.

Moreover, the methods disclosed by SHUBER are based on PCR amplification, gel electrophoresis analysis and ethidium bromide staining. SHUBER mentioned other staining possibilities but considered this variation as a simple alternative without contemplating the possibility of important improvements.

Thus, SHUBER stands in contrast to the claimed method. The claimed method is based on fluorescence analysis which determines an improvement in test sensitivity and specificity for the detection of colorectal cancer.

The use of ethidium bromide staining and quantification results in a poor discrimination between healthy individuals and colorectal cancer patients. In fact, gel staining does not permit an accurate quantification of PCR amplification and only a few classes can be obtained: null, low, medium or high levels of PCR amplifications (Gastroenterology 119:1219-1227, 2000).

In this way, colorectal cancer patients with fecal DNA levels only slightly higher than those of healthy individuals fall into the same category as healthy individuals, with a consequent misclassification of a vast number of colorectal tumors and a lower method sensitivity.

The quantitative approach described in the claimed method determines a continuous scalar evaluation of each case, permitting the correct identification of patients with a PCR amplification level only slightly higher than that of healthy individuals.

This allows one skilled in the art to better evaluate PCR amplification levels and to determine more accurately the best cut-off to discriminate between healthy subjects and patients.

With this fluorescent method, it is believed that a significant improvement in sensitivity with respect to the one described in Shuber's document was obtained. As described in the article published in 2004 in Neoplasia, when comparing the "ethidium bromide method" with "fluorescent methods", a sensitivity improvement of about 30% was obtained (Neoplasia, 6:536-540, 2004). In this regard, not only does SHUBER fail to anticipate the claimed invention but it is believed that the claimed invention provides an unexpected and unobvious sensitivity improvement.

SHUBER considers the fluorescent or radioactive labels as mere alternatives to ethidium bromide staining, without taking into account the improvement that different labeling could determine, as shown in our paper published in Neoplasia (2004;6:536-540) and discussed above. Indeed, fluorescent and radioactive labels, in terms of correct quantification of long DNA are not equivalent and radioactive labeling does not permit the same quantitative evaluation that can be achieved with fluorescent labeling.

Thus, it is believed that SHUBER fails to anticipate or render obvious the claimed invention.

Claim 2 was rejected under 35 USC 103(a) as allegedly being unpatentable over SHUBER in view of ZHOU. Claim 8 was rejected under 35 USC 103(a) as allegedly being unpatentable over SHUBER in view of KMIEC, KMIEC and ALBERTSON, and BUCK. These rejections are respectfully traversed.

None of the publications of ZHOU, TIAN, KMIEC, KMIEC and ALBERTSEN, or BUCK remedy the deficiencies of SHUBER for reference purposes.

While ZHOU is cited for the proposition that it would have been obvious to use fluorescein to study colorectal cancers, ZHOU does not disclose or suggest using fluorescein with any of the steps recited in the claimed invention. Accordingly, it is believed that ZHOU fails to remedy the deficiencies of SHUBER for reference purposes.

As to KIMIEC, ALBERTSON, and BUCK, the official Action acknowledges that the publications do not disclose the primer sequences of SEQ ID NOS 9-10 and 13-16 (see claims 7, 8, and 13). Rather, the Official Action contends that primers are obvious over the cited reference as they are homologs of the sequences disclosed by KIMIEC, ALBERTSON, and BUCK. Applicants respectfully disagree.

The claimed primers do not "simply represent structural homologs". Moreover, it is believed that the reliance of the Official Action on *In re Deuel*, 34 USPQ 2d 1210 (Fed Cir. 1995) is improper. Indeed, not one of the recited primers would have been obvious as there is nothing in the publications that would have lead one skilled in the art to the particular primer or indicate that that any of the primers should be prepared. Indeed, none of the publications teach a specific, structurally-definable compound in accordance with the primers recited in the claims or suggest the specific molecular modifications necessary to achieve the claimed invention.

Applicants also point out that the BUCK method refers to sequencing analysis and not to the quantification of DNA levels. The performance and correspondence required for a sequence analysis is different from those needed for a quantification study. For sequencing analysis, the quantity of PCR amplification is not such a stringent or selective limitation. The main factor to be considered is the specificity

of the PCR amplicon and purity in order to obtain a good sequencing result. For DNA quantity evaluation, in addition to having a good PCR amplicon quality, it is also important to have a good linearity of the amplification curve and a good correlation with cycle numbers and cycle temperature.

Thus, contrary to the assertions of the Official Action, not all primers produce the same results. In this regard, applicants present additional data using different primers that have been designed with the same genomic regions in mind but simply do not produce comparable results in terms of specificity of colorectal cancer detection.

Table 1 shows the results, in terms of FL-DNA (Fluorescence long DNA) values expressed as nanograms (see also patent description), obtained by two different approaches:

i) the primers as recited in the claims, and

ii) a new series of primers designed with similar genomic regions.

The results show that the two sets of data are not comparable, indicating that the use of a selected series of primers determine different FL-DNA values. This difference is also indicative of a different ability to discriminate between cancer patients and healthy subjects. In fact, the aim of the FL-DNA method is to identify cancer patients with the highest sensitivity and specificity possible.

Tables 2a/b show the sensitivity and specificity of the two different approaches. In Table 2a, using the primers recited in the claimed invention, it is possible to obtain good sensitivity and specificity with different cut-offs (see, for example, 10-15 or 20 ng cut-offs). Conversely, using the other primers (Table 2b), sensitivity and specificity suffers. This problem does not allow one to identify an accurate (high sensitivity and specificity) cut-off for colorectal cancer detection.

Table 1

Results of FL-DNA analysis using different primers

Tumor no.	FL-DNA (ng) "Claimed" primers	"New" primers
1	34	33
2	41	9
3	19	12
4	42	12
5	10	10
6	9	3
7	35	3
8	21	8
9	96	6
10	77	11
Healthy donor no.		
1	9	6
2	13	9
3	13	7
4	14	13
6	4	13
6	15	2
7	25	12
8	17	4
9	0	2

Table 2a

Sensitivity and specificity of FL-DNA analysis with "claimed" primers

Cut-off (ng)	Sensitivity no. cases	%	Specificity no. cases	%
10	9	90	7	30
15	8	80	3	70
20	7	70	2	80
25	6	60	2	80
30	6	60	1	90

Table 2b

Sensitivity and specificity of FL-DNA analysis with "new" primers

Cut-off (ng)	Sensitivity no. cases	%	Specificity no. cases	%
5	8	80	6	40
10	5	50	3	70
15	1	10	0	100
20	1	10	0	100
25	1	10	0	100

Methods

DNA extraction

Genomic DNA was extracted from 10-20 mg of feces obtained from the spiral groove of the commercially available

immunochemical Fecal Occult Blood Test (FOBT (iFOBT OC-Sensor, Alfa Wassermann).

1) One milliliter of TE buffer is added to a 1.5-ml test tube and used to wash the stool attached to the spiral groove.
2) Centrifuge for 15 minutes at 5,000 g. Transfer the supernatant to a clean sterile test tube and add 155 µl of ammonium acetate 7.5 M and 930 µl of ethanol 100%. Mix and centrifuge for 15 minutes at 5,000 g.
3) Extract DNA from the pellet using QIAmp DNA stool kit.

Amplification and FL-DNA analysis is performed in the same way as described in the patent application, the final result is normalized to the data obtained from 4 gr of stool multiplying the results by 3. This value was obtained by evaluating the results of FL-DNA obtained from 4 gr and 10-20 mg of stool in the same samples. A similar normalization value could be obtained using the same approach and quantities other than the standard 4 gr.

Fluorescence long DNA (FL-DNA) analysis

The FL-DNA analysis using the primer described in the claims was performed as previously described in the patent application.

For the new set of primers the p53 sequences were as follows: exon 5: 5A _FAM-CAA CTC TGT CTC CTT CCT CTT CC and 5B_ AAC CAG CCC TGT CGT CTC T; exon 6: 6A_CAG GCC TCT GAT TCC TCA CT

and 6B_HEX-CTT AAC CCC TCC TCC CAG AG; exon 7: 7A _FAM-TCA TCT TGG GCC TGT GTT ATC and 7B _TGG AAG AAA TCG GTA AGA GGT G; exon 8: 8A GGG ACA GGT AGG ACC TGA TTT and 8B_HEX-TAA CTG CAC CCT TGG TCT CC.

APC sequences were as follows: fragment 1: 1A_CCC TAG AAC CAA ATC CAG CA and 1B_ HEX-CAT TC ACT GCA TGG TTC AC; fragment 2: 2A_ FAM- GTG AAC CAT GCA GTG GAA TG and 2B_CAC TCA GGC TGG ATG AAC AA; fragment 3: 3A_AAG AAG CTC TGC TGC CCA TA and 3B: HEX- GTG AAC CAT GCA GTG GAA TG; fragment 4: 4A_ FAM- GTC AAT ACC CAG CCG ACC TA and 4B_ GTC AAT ACC CAG CCG ACC TA.

The p53 exons 5 to 8 and fragments 1 to 4 of APC were amplified in a final volume of 25 ml containing 2 ml of stool DNA, 0.4 mM of each primer, 200 mM of deoxynucleotide (Takara Bio Inc), 1X reaction buffer with 3.5 mM MgCl2 (Qiagen), and 1 U of Taq polymerase (Qiagen). The reaction mixture was subjected to 32 cycles: 60 s at 94°C and then 60 s at 58°C f followed by incubation at 72°C for 60 s. for all fragments. Primers used were end-labeled with fluorochromes provided by Applied Bioystems.

DNA from each sample was quantified on a standard curve of genomic DNA (1, 2, 5, 10 and 20 ng) normalized to 100, and expressed as nanograms.

Electrophoresis was carried out using a 3100 Avant Genetic Analyzer (Applied Biosystems) equipped with GeneScan Analysis 3.7. The final FL-DNA value was obtained by analyzing

the fluorescence intensity of each sample-specific PCR product. The quantification of each sample was calculated by reference to a standard curve (1, 2, 5, 10, and 20 ng) of genomic DNA and expressed as nanograms.

The DNA samples were obtained from a small amount of feces (10-15 mg) and for this reason the FL-DNA value obtained using the claimed primers was normalized to the data obtained from 4 gr of stool multiplying the results by 3.

Thus, contrary to the assertions of the Official Action, not all primers produce the same results. Accordingly, applicants respectfully submit that the above-identified publications, alone or in combination, fail to disclose or suggest sequences recited in the claims (e.g., see claims 7, 8, and 13).

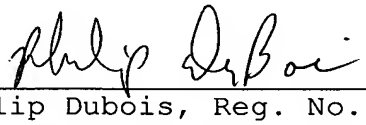
In view of the present amendment and foregoing Remarks, therefore, applicants believe that the present application is in condition for allowance at the time of the next Official Action. Allowance and passage to issue on that basis is respectfully requested.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any

overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

YOUNG & THOMPSON



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